

ABSTRACTS FROM THE ORIGINAL PAPERS.

Studies on Castor-bean Lipase. I.

I On the Vessel for Estimating the Activity of the Lipase.

By Etsuo TAKAMIYA.

(From the Biochemical Laboratory, Department of Agriculture, Kyushu
Imperial University, Fukuoka, Japan.)

(Received Oct. 10th. 1925.)

It was examined in this study what influence had the size of estimating vessels upon the estimation of the activity of castor-bean lipase.

The lipase preparation applied for this study was obtained as white fine powder, from castor-beans after removing their husks by hand, extracting the kernels with petroleum-ether. The method of estimating for the activity was similar to that of R. Willstätter (Zs. f. phys. Chem., **134**, 177, 1924.) except that a cylindrical bottle with a glass-stopper, a definite diameter, and a flat bottom was used, under the reason stated below, as an estimating vessel.

The results of experiments are concluded as follows :-

1. The castor-bean lipase splits the substrate at the contact surface between the oil-layer containing the lipase preparation and buffer solution-layer.
2. Even under the same conditions, the degree of hydrolysis of the substrate by the lipase preparation varies according to the size of estimating vessels i. e. to the area of the contact surface between the two layers.
3. The larger the area of the contact surface, the greater is the degree of hydrolysis of the substrate by the lipase preparation.
4. The activity of the lipase preparation of n mg. in 1 sq. cm. of the contact surface is equal to that of 1 mg. each in n sq. cm.

As it is obvious from the above-mentioned statement that the activity is effected by the area of the contact surface, the following items for estimating

vessels were proposed.

1. It is advantage to use a cylindrical bottle with a glass-stopper and a flat bottom as the estimating vessel in order to keep a constant contact surface during the reaction of the enzyme.

2. A vessel with a definite diameter should always be used for the comparison of the enzyme-action.

3. It is desirable to record the diameter of the estimating bottle in each experiment.

On the Nutritive Value of Synthetical Fats Containing Fatty Acids of Uneven Number of Carbon Atoms.

By Jun-ichi OZAKI.

(From the Biochemical Laboratory, College of Agriculture, Imperial University of Tokyo).

(Received Nov. 20th. 1925.)

It is a well known fact that fatty acids occurring in natural fats generally contain even-number carbon atoms. K. Takahashi (J. Chem. Soc. Japan. Vol. 43 No. 3) in our laboratory has recently prepared a series of triglycerides from chemically pure fatty acids, and found that, by the feeding experiments of albino rats with them, those fats consisting of lower fatty acids from 2-12 carbon atoms were better utilized by the animals than those of higher fatty acids such as palmitic, stearic, or oleic acids, and also that the mixture of free fatty acids and glycerin was inferior to the corresponding glyceride in nutritive value. Such a difference was more pronounced when the rats were fed on the artificial diet poor in vitamin-A: i. e. solid fats such as palmitin, stearin etc. were very difficultly assimilated or sometimes even retarded the animal growth unless an enough amount of vitamin-A was not given with them; while the lower members such as caprylin, caprin, laurin or myristin etc. could be more easily utilized and resulted a good animal growth even if the diet was deficient in vitamin-A. This evidence is especially interesting on account of that butter fat consists of mostly lower glycerides: those of from butyric to lauric acids; and those of oleic and palmitic acids, etc. are present in it only in negligible quantity. Takahashi further observed that the rats could attain the normal

growth with a fat-free diet, provided other nutritive factors were supplied in adequate proportion. Thus, the fat can be considered as not an indispensable food factor, at least, for a certain kind of animals.

The author has carried out a series of feeding experiments with the synthetical fats which were prepared from fatty acids containing uneven-number carbon atoms for the purpose of determining whether they could equally be well utilized by animals as in the case of those of even-number carbon atoms.

From the clinical stand-point of diabetes this subject is also of some interest, for it is now believed by some authors that synthetic margarin (intervin) prepared from margarinic acid of 17 carbon atoms may be recommended for diabetes; and the synthetic margarin does not, according to these authors, produce the noxious "aceton bodies", which fact can be easily understood by the Knoop's " β -oxidation theory". On the other hand, others think another noxious product such as propionic or valerianic acid etc. might be produced from "intervin" instead of "aceton bodies". Therefore, careful feeding experiments with pure synthetical fats are absolutely necessary for the solution of the question.

The fatty acids used by the author for the synthetical preparation of fats were formic, propionic, isovaleric, heptolic, palargonic, undecylic, undecylic, tri-decylic, pentadecylic and margarinic acids. All these, except propionic and isovaleric acids, were prepared by the author and they are carefully purified, and their melting- and boiling-points, mean molecular weights etc. were all examined thoroughly.

The preparation of triglycerides from these fatty acids was carried out as follows:— A calculated amount of pure glycerin was mixed with a little excess of fatty acid; to this mixture was added 5% of Twitschell's reagent, and heated at 100°C for about five hours, stirring the mixture occasionally in order to expell off the water produced by the reaction; the mixture was dissolved in ether when the reaction was completed and carefully washed with dilute alkali, acid and water, successively. The etherial solution, after dehydrating with dry sodium sulphate, was evaporated, and the residue was either subjected to fractional distillation in vacuum or recrystallized from alcohol or ether, according to the nature of fats.

The basal diet for the albino rats consisted of 80% starch, 15% meat protein, 5% McCollum's salt mixture, 3% commercial "Oryzanin" and 0.1% "Biosterin". Young rats were previously fed on a limited quantity of the above diet (9 grams in dry matter for a rat per day), having each one been kept in

a separate cage; and thus the animals grew at first to a certain degree, but they soon reached to the equilibrium state when the given amount of the diet was just sufficient for the maintenance of the body weight. At this point the synthetical fats tested were added to the above diet in the proportion of 5, 10, or 20%. If the fats intaken were utilized by the rats, the animals would begin to grow, but in case they were noxious or quite useless for the animals the body weight would either decrease or at least remain constant. So, by plotting the body weight in curves the relative nutritive value of these fats can be easily shown. For control's sake, the parallel experiments with the fats of even-number carbon atoms such as acetin, butyryn, caprylin etc. were carried out under the same condition as above. The results obtained in this way are given below according to the order of their nutritive value.

1. By adding 5% fats to the basal diet:

palmitin, caprylin, acetin, heptylin, butyryn, undecylin, nonylin, pentadecylin, tridecylin, margarin, stearin, undecylein, propionin, valerin.

2. By adding 10% fats:

caprylin, heptylin, butyryn, pentadecylin, palmitin, tridecylin, stearin, acetin, nonylin, margarin.

3. By adding 20% fats:

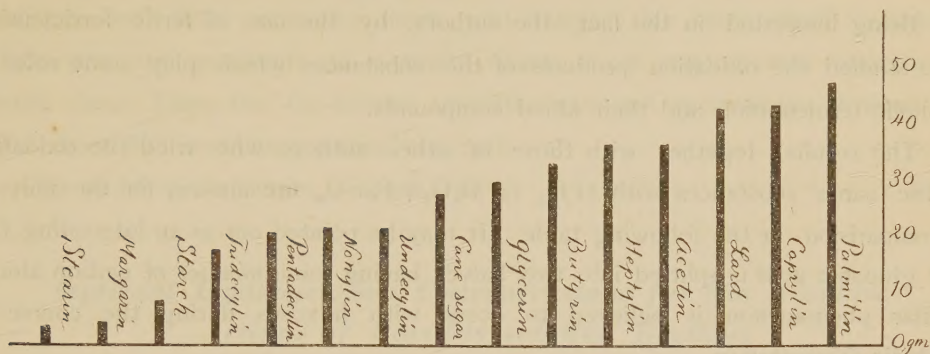
tridecylin, pentadecylin, heptylin, stearin.

Among these, propionin, isovalerin and undecylein exhibited a noxious effect when 5% of these was added to the basal diet given. By decreasing this additional amount to 2.7%, propionin and undecylein were found to be nutritious, while isovalerin was still noxious. It is found by the author that the last substance was the most noxious one among the above fats. Undecylin prepared by means of the catalytic reduction of undecylein was proved to be nutritious when an extent of 5% of this substance present in the diet. Therefore the noxious property of undecylein may be due to the presence of double linking in its molecule.

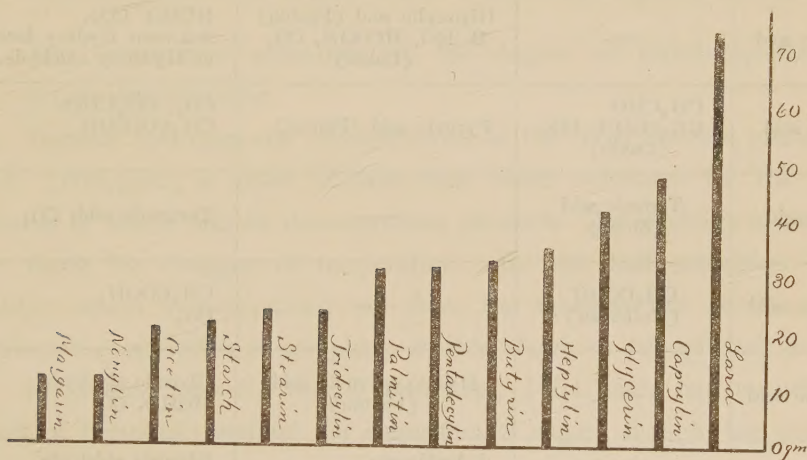
The experimental results given above are considered as most probably presenting only an approximate value, for the animal growth is much influenced not only by the room temperature, but also by the different seasons in which the feeding experiments are made.

The author also observed that the addition of vitamin C in these experiments gave not any noticeable effect upon the results.

*Showing the increase of body weight after adding 5%
of sample to the basal diet.*



*Showing the increase of body weight after adding 10%
of sample to the basal diet.*



Oxidative Nature of Iron Compounds. Part II.

Ferric ferricyanide as an oxidizing agent of organic compounds.¹

By Bunsuke SUZUKI and Tsutomu MARUYAMA.

(Received, Nov. 12, 1925)

Ferric ferricyanide is an good oxidizing reagent for α -hydroxy- and α -ketonic acids of aliphatic series, giving aldehydes and ketones as products, upon which as well as upon alcohols, ketones, sugars and α -amino acids, the reagent has no

power of reacting. To some extent, therefore, the action of the reagent resembles that of "carboxylase" of Neuberg.

Being interested in the fact, the authors, by the use of ferric ferricyanide, have studied the oxidation products of the substances which play some roles in alcoholic fermentation and their allied compounds.

The results, together with those of other authors who tried the oxidation of the same substances with H_2O_2 or $\text{H}_2\text{O}_2 + \text{FeSO}_4$, are shown, for the purpose of comparison, in the following table. It may be pointed out as an interesting fact that gluconic acid is splitted into two halves having same number of carbon atoms. Similar phenomenon is believed to occur with hexoses during the course of alcoholic fermentation.

	H_2O_2	$\text{H}_2\text{O}_2 + \text{Fe}''$	$\text{FeFe}(\text{CN})_6$
Glycolic acid	—	Glyoxylic acid (Fenton) HCHO , HCOOH , CO_2 (Dakin)	HCHO , CO_2 , unknown Hydroxy-ketone, or Hydroxy aldehyde.
Lactic acid	CH_3CHO CH_3COOH , CO_2 (Dakin)	Pyruvic acid (Fenton)	CO_2 , CH_3CHO , CH_3COCOOH
Malic acid	Tartaric acid (Zinno)	—	Tartronic acid, CO_2
Pyruvic acid	CH_3COOH (Holleman)	—	CH_3COOH , CO_2
Glyceric acid	—	Hydroxypyruvic acid (Fenton)	Glycolic aldehyde, HCHO , CO
Gluconic acid	—	d-Arabinose Oxy-gluconic acid HCOOH , CH_2OH	Glyceric aldehyde, Glycolic aldehyde.

On the Preparation of Ca-lactate from the Still-residue of Biityû.

By Syoosuke TANAKA.

It has been published that the fresh still-residue of Biityû contains about 1.5% of lactic acid cheffy. Filtering a large quantity of fresh still-residue of

Biityû as fast as possible with filter press not to be produced other acids by bacteria, I neutralised with Ca-hydroxide and obtained Ca-lactate. Filter off the precipitate with filter press once more, and transfer this clear filtrate in a large surface pun and evaporate to a small volume about one seventh blowing by electric fan. Then the Ca-lactate separates out and recrystallise it from hot water about 50° containing animal charcoal.

The yield of Ca-lactate is about 2%.

***Optimal Hydrogen-ion Concentrations for the Amylase
Actions of Bacillus subtilis, Bacillus
mesentericus and Aspergillus oryzae.***

By Shokichi KATO and Shunzi KAKEGAWA.

- I. The quantitative estimation of the degree of starch-liquefaction and starch-dextrinization.
- II. Optimal hydrogen-ion concentrations in the three reaction phases.

[I]. $(\text{NH}_4)_2\text{SO}_4$ is more suitable than other sulphates for the fractional precipitation of starch and its decomposition products. Its solubility is not affected so very much by changes of temperature. By the half saturation with this $(\text{NH}_4)_2\text{SO}_4$, starch is precipitated out from the solution, but soluble starch is not. When the saturation is complete, soluble starch is precipitated, but dextrin is not. In smaller amounts than $\frac{1}{4}$ saturation, $(\text{NH}_4)_2\text{SO}_4$ has no influence on the result of Bertrand's method for estimation of sugar, though the oxidation of copper hydroxide is hastened more or less and the color of cuprous oxide is darkened.

The method applied in this study for the estimation of the degree of starch-liquefaction and starch-dextrinization is outlined as follows:

A calculated amount of $(\text{NH}_4)_2\text{SO}_4$ for half saturation (liquefaction) or complete saturation (dextrinization) at room temperature is taken in a 50c.c. volumetric flask. To this 25c.c. of starch digesting solution is poured in. It is filled up to the mark with water, mixed and then allowed to stand. After 1-2 hours the solution is filtered through dry paper. 25c.c. of the filtrate of which a small portion has been removed, is taken into a 100c.c. volumetric flask, 25c.c. of 8% HCl is added. And it is heated at 100°C for 3 hours, when cold the

solution is neutralized until weak acidic reaction results, then filled up to the mark. This is analysed by Bertrand's method.

[II]. The hydrolysis of starch by amylases is, obviously, a consecutive reaction. A question arises, therefore, whether the influences of hydrogen-ion concentration on the action of any amylase in different reaction phases are the same or not. It seems that no definite answer has yet been given as to this question, which is the main object of this study.

On the other hand, somewhat disaccordant values ranging from 4.0 to 5.2 have been given as the optimal pH for the action of "Koji-amylase" by some authors. And on the action of bacterial amylases, there has been done few studies concerning the influence of hydrogen-ion concentration.

The authors determined the optimal hydrogen-ion concentration for three reaction phases, i. e., starch-liquefaction, starch-dextrinization and starch-saccharification, by means of the method above mentioned and Bertrand's method. Iodine method was used also in comparison with the the new method.

The amylases were prepared from the cultures of *Asp. oryzae* and *subtilis-mesentericus* bacteria on cooked wheat bran.

The optimal pH values obtained are as follows:

	Liquefaction	Dextrinization		Saccharification
		New method	Iodine method	
<i>Bac. subtilis</i>	—	—	6.7	6.7
B 6 (Isolated from ropy boiled-soybean)	6.7	—	—	6.7
<i>Bac. mesentericus vulg.</i>	—	—	6.7	—
R 32 (Isolated from ropy boiled-rice)	6.75	6.75	6.7	6.6
av.	6.7	6.7		6.7
<i>Asp. oryzae</i> ("Sake-koji" mold)	4.3	4.2	4.3	4.8
<i>Asp. oryzae</i> ("Tamari-koji" mold)	4.35	—	4.4-4.4	4.7-4.8
av.	4.3-4.4	4.3-4.4		4.7-4.8

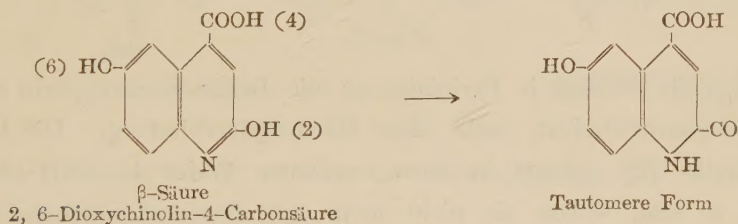
The optimal pH values for bacterial amylases agree at about 6.7, throughout the three reaction phases. For "Koji-amylase", the value is higher in saccharification phase than in the others. This difference can not be judged as due to experimental errors. It must be remarked that the two enzyme theory of amylase can not be supported by this fact, because amylolytic reaction is consecutive in itself.

**Über die Konstitution der durch Hydrolyse des
Rohoryzanins entstehenden β -Säure
(Dioxy-chinolincarbonsäure)**

II. Mitteilung.

von Yoshikazu SAHASHI.

In der ersten Mitteilung⁽¹⁾ über die β -Säure $C_{10}H_7NO_4$ hat der Verfasser die Anwesenheit von zwei Hydroxyl und einer Carboxylgruppe in ihrem Moleküle festgestellt. Ferner erhielt er Chinolin durch Zinkstaubdestillation. So ist diese Säure als eine Dioxychinolin-carbonsäure aufzufassen. $C_9H_4N(OH)_2COOH$. Aber die relative Stelle der Hydroxyl und Carboxylgruppe blieb unentschieden. Daher hat der Verfasser zur Aufklärung dieser Frage das Studium dieser Substanz fortgesetzt und kam zum Schluss, dass die β -Säure wahrscheinlich eine 5·6-Dioxychinolin-4-Carbonsäure ist, welche leicht in ihre tautomere Form in folgender Weise übergeht:



Diese Annahme wird durch folgende Beobachtungen unterstützt:

(1) Die β -Säure bildet durch Acetylieren mit Essigsäureanhydrid und Natriumacetat ein Diacetylderivat.

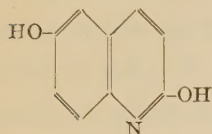
(2) Die β -Säure bildet durch Methylieren mit Dimethylsulfat und Alkali in üblicher Weise ein Dimethylderivat. Das letztere bildet ein Baryumsalz von der Zusammensetzung $[(CH_3)_2C_9H_4NO_2 \cdot CO_2]_2Ba$ beim Kochen mit Baryumcarbonat in wässriger Lösung. Die β -Säure gibt auch in derselben Weise ein Baryumsalz $(C_9H_6NO_2CO_2)_2Ba$.

(3) Wie oben erwähnt liefert die β -Säure durch Zinkstaubdestillation Chinolin. Die Ausbeute war aber zur exakten Schlussfolgerung, dass es den Kern der β -Säure bildet, zu gering. Durch Erhitzen der β -Säure mit Zinkstaub im

(1) Bioch. Zeitsch. 159, Heft 3/4 221-234.

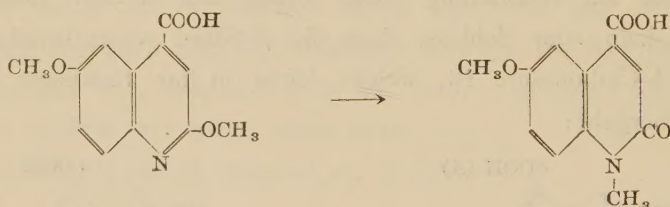
Wasserstoffstrom über 400° wurde etwas mehr Chinolinerhalten obwohl seine Menge viel geringer als die berechnete Menge war.

(4) Erhitzt man die β -Säure über 400° , so spaltet sich Kohlensäure ab und es sublimieren feine gelbe Nadeln vom F. P. 310° die als 2·6-Dioxychinolin

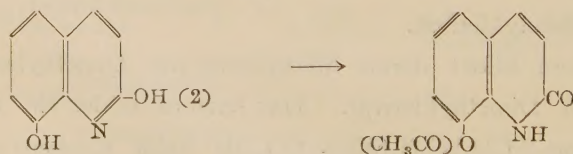


identifiziert wurden. Die Ausbeute dieser Substanz ist auch schlecht. Aber die Existenz des Chinolinkerns in der β -Säure ist dadurch viel wahrscheinlicher geworden.

(5) Im Dimethylderivat der β -Säure findet sich eine Methylgruppe als Methoxyl, weil sie durch Erhitzen mit Jodwasserstoffsäure nach Zeisel's Methode leicht abgespalten wird, während die andere-N-Methylgruppe bildet, die erst beim Erhitzen mit konzentrierter Jodwasserstoffsäure und Jodammonium auf 300° abgespalten wird. Deshalb nimmt der Verfasser an, dass ein Methyl an Stelle (2) in N-Methyl umgewandelt ist:



(6) Wird die β -Säure in Pyridinlösung mit Benzoesäureanhydrid erhitzt, so gibt sie Monobenzoylderivat, nicht aber Dibenzoylverbindung. Die Hydroxylgruppe in Stelle (2) scheint in oben erwähneter Weise in $-NH-CO-$ Gruppe umgewandelt zu sein, sodass sie nicht mehr mit Benzoesäureanhydrid reagiert. Eine Analogie fall findet man beim 2·8-Dioxychinolin, welches nach J. Diamant⁽¹⁾ mit Essigsäureanhydrid nur ein Monoacetylderivat bildet.

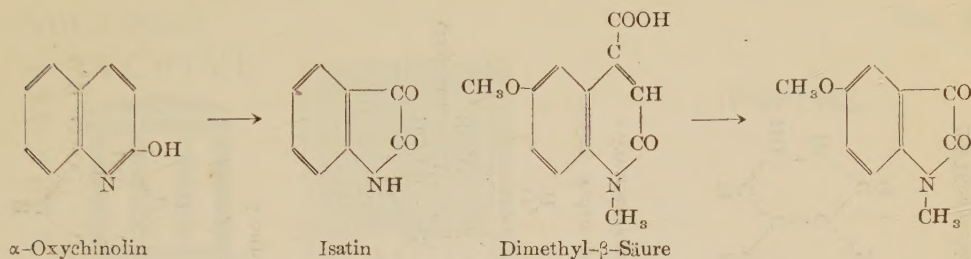


(7) Das Dimethylderivat der β -Säure in kalter alkalischer Lösung mit $KMnO_4$ behandelt, liefert rote Krystalls, die als ein Isatin-derivat von folgender Konstitution identifiziert wurden. Da Friedländer und Ostermeier⁽²⁾ aus α -Oxychinolin in derselben Weise Isatin erhielten, kann man sich die Reaktion infol-

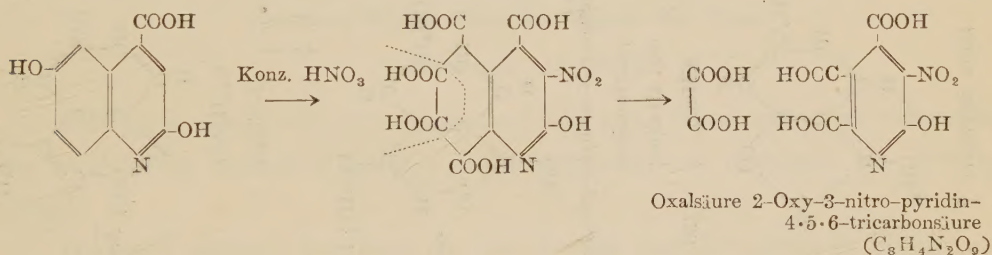
(1) M. 16 765-767.

(2) B. 15 332.

gender Weise vorstellen :

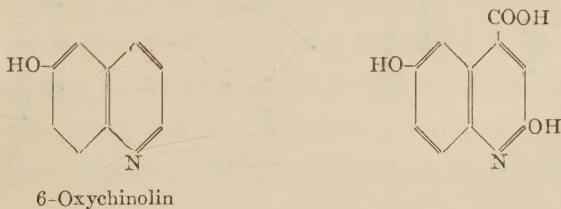


(8) Mit starker Salpetersäure erhitzt, lieferte die β -Säure ausser Oxalsäure eine Nitroverbindung von der Formel $C_8H_4N_2O_9$, die zuerst vom Verfasser als Bernthen's Juglonsäure angenommen wurde. Später hat er aber durch Zinkstaubdestillation derselben Pyridin erhalten. Höchst wahrscheinlich handelt es sich um 2-Oxy-3-nitropyridin-4.5.6-tricarbonsäure. Ihr Bildungsweise ist in folgender Weise zu erklären.

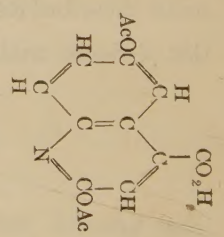
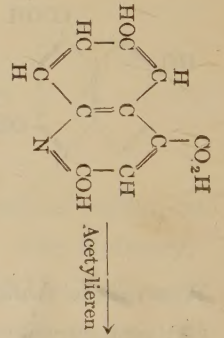
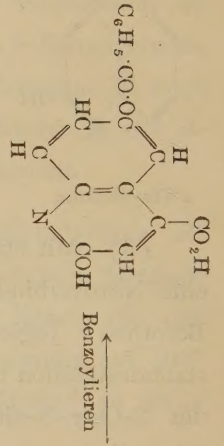


Wenn diese Annahme zutrifft, so muss die Carboxylgruppe der β -Säure die Stelle (4) einnehmen.

(9) Die β -Säure gibt ziemlich starke Thalleiochin reaktion. Da 6-Oxychinolin dieselbe Reaktion gibt, kann man annehmen, dass eine Hydroxylgruppe der β -Säure analog an Stelle (6) haftet.



(10) Die obigen Beobachtungen lassen sich in folgender Weise schematisch darstellen :

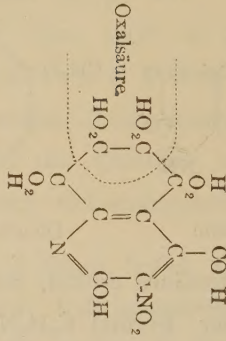


6-Benzoyloxy-carbostyryl-
4-carbonsäure
Konz.

$\text{C}_{10}\text{H}_7\text{NO}_4$
 β -Säure

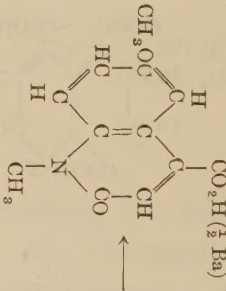
Me_2SO_4

2,6-Diacetoxy-quinoline-
4-carbonsäure

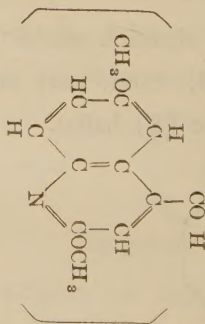


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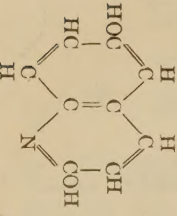
p-Methoxy-N-methyl- α -quinolon-
 γ -carbonsäure



2,6-Dimethoxy-quinolin-
4-carbonsäure



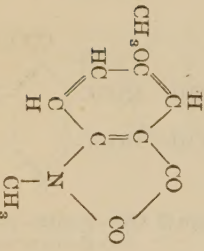
2-Oxy-3-nitro-pyridin-
4,5,6-tricarbonsäure



+ CO₂

2,6-Dioxy-quinolin ?

Iatin derivative ?



p-Oxy-N-methyl- α -quinolon-
 γ -carbonsäure

